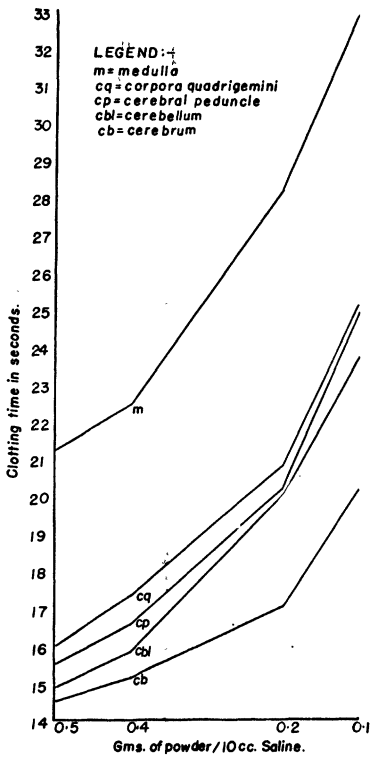

FIG. 1. Comparison of the thromboplastic potency of the various brain parts in different concentrations.

was also evident when greater concentrations of powder were used.

Fig. 1 presents the data obtained with the pooled brain parts of a dozen rabbits used in the various concentrations. It is seen that the clotting times of the 0.1 g/10 ml extracts correspond well to the means of Table 1, and that the difference between medulla and the other morphological parts of the brain is also evident at the higher concentrations of thromboplastic suspensions.

It is too early to attribute general significance to the data presented here; such analyses must await further studies. An immediate result, however, has been the improvement of the thromboplastic preparations used in the prothrombin determination in the clinical laboratory. These are now prepared minus the medulla. Phenol to a final concentration of 0.25% is

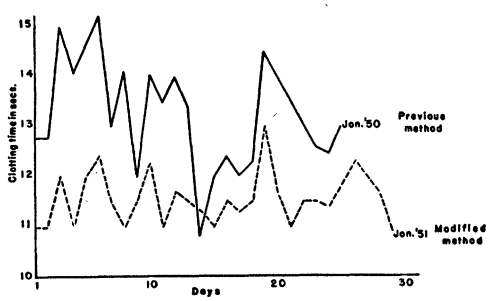

FIG. 2. Comparison of routine control prothrombin times obtained with the former and with revised thromboplastic preparations.

also added to the suspensions to prevent bacterial growth, which seriously reduces the potency of the thromboplastic suspensions (*2*). The behavior of these preparations has been gratifying: activity has been increased, and variation between normal controls has been reduced (Fig. 2). Normal control times range between 10–13 sec and are largely between 11–12 sec. The resulting preparations have also been very stable; large amounts of such suspensions have been kept in the refrigerator for a month, with portions removed for daily routine use, with no apparent deterioration.

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The Viscosity of the System Methanol-Toluene

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The viscosity composition curves of many binary liquid systems have points of inflection, and when the components are of nearly equal viscosity they may even exhibit maxima and minima where the viscosity is respectively greater or smaller than that of either pure component. Such curves have been reported for the systems formamide-isobutyl alcohol and formamide-isoamyl alcohol (*1*).

Viscosity data on the system methanol-toluene at 20° C lead to a strikingly similar curve (Fig. 1). The

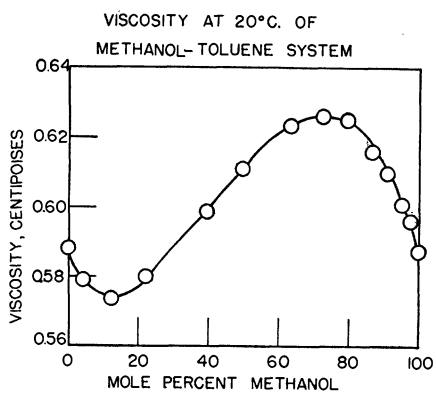

FIG. 1.

density determinations were not precise enough to show whether there is any dilatation when mixing small amounts of methanol with toluene, but showed a definite contraction when the solution contained more than 20 mole % methanol (Fig. 2).

The methanol used for these experiments was Baker's C. P. Analyzed Absolute Methanol, assay (by vol) 99.5%. The toluene was bought from Central Scientific Company and was labeled "Toluene for Technical Use." These materials were purified by

distillation through a Hempel-type column packed with glass beads using a reflux ratio of 3:1.

The viscosities were determined with a Fisher-Irany viscometer, and the densities with a bicapillary pycnometer, as described by Lipkin *et al.* (*2*). The water-bath temperature was controlled within 0.05° C.

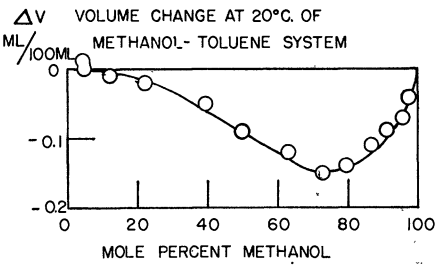

FIG. 2.

The specific heat, heat of mixing, and volume change on mixing of this system at 25° C and 35° C have been studied by Mason and Washburn (*3*), who observed contraction for all compositions and concluded that the associated pure methanol undergoes dissociation when it is dissolved in toluene and that simultaneously there is solvation of the methanol by the toluene.

Similar conclusions were reached by Washburn and Lightbody (*4*) when investigating the volume change on mixing of this system and of other aliphatic alcohols dissolved in benzene or toluene. They also reported contraction throughout the composition range for the methanol-toluene system.

Bushwell, Deitz, and Rodebush (*5*) studied the infrared absorption spectra of methanol and found that the association in concentrated methanol solutions is caused by hydrogen bonding.

Harms (*6*) has discussed the volume changes in this kind of system by assuming that the alcohol exists partly as association polymers of various chain lengths, produced by repeated reaction steps obeying the law of mass action, each reaction step having the same equilibrium constant. According to these views, the observed volume change is the difference between the dilatation caused by depolymerization and the contraction caused by solvation.

The present data show that the viscosity cannot be a function of the volume change alone, but that the reduction in chain length of the association polymers must also contribute to the decided lowering of viscosity when a small amount of methanol is dissolved in toluene.

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Phosphogalactoisomerase[1]

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Caputto and his co-workers (*1*) have shown that the first stages in the fermentation of galactose by the adapted yeast *Saccharomyces fragilis* may be represented by the following equations:

Galactose + ATP ⟶ Galactose-1-phosphate (I)
Galactose-1-phosphate ⟶ Glucose-1-phosphate (II)
Glucose-1-phosphate ⟶ Glucose-6-phosphate (III)

Reaction I is catalyzed by an enzyme, galactokinase, which was separated from the yeast extracts in a partially purified form (*2*). Reaction III is the well-known phosphoglucomutase reaction, which requires diphosphoglucose as a coenzyme (*3*). The enzyme associated with reaction II was not isolated but was detected in the crude yeast extracts by virtue of its requirement for the newly described coenzyme, uridinediphosphoglucose (UDPG). When this coenzyme was added to extracts of the macerated *S. fragilis* cells, an increased rate of formation of glucose-6-phosphate from galactose-1-phosphate was observed (*4*).

In the course of our investigations of the metabolism of galactose in animal tissues we have used the washed cells of *S. marxianis* to remove galactose from tissue extracts and have had occasion to investigate the above reactions with both *S. fragilis* and *S. marxianis*. We have confirmed the essential features of the reaction mechanisms proposed by the Argentine investigators and have extended their observations by the isolation of the enzyme which catalyzes the transformation of galactose-1-phosphate into glucose-1-phosphate. We suggest that this enzyme be named *phosphogalactoisomerase*.

Forty-eight-hr cultures of *S. marxianis*, grown in a yeast extract–galactose media, were harvested and washed with water. The cells were dried *in vacuo* and were then ground to a fine powder. This material was extracted with a phosphate buffer at pH 7.6 and yielded a solution which was rich in the three above-mentioned enzymes. When this extract was acidified to pH 5, a precipitate was formed which was collected and suspended in water. Clarification of this preparation by centrifugation at 8,000 rpm yielded a solution which contained most of the phosphogalactoisomerase but no galactokinase and no phosphoglucomutase.

The enzyme is inactive in the absence of the UDPG coenzyme. However, when this coenzyme is added to a mixture of the enzyme and galactose-1-phosphate, the substrate is converted into a nonreducing phosphorylated hexose which has been identified as glucose-1-phosphate. A typical protocol is shown in Table 1. Since the determinations of glucose-1-phosphate and

[1] This investigation was supported by a grant from the Division of Research Grants of the National Institutes of Health, USPHS.